

The epidemiology of *Neisseria gonorrhoeae* isolates in Dakar, Sénégal 1982-1986: antimicrobial resistance, auxotypes and plasmid profiles

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Abstract

A total of 460 *Neisseria gonorrhoeae* isolates from patients seen at three clinics in Dakar, Sénégal, 1982-1986, have been investigated. In this period a significant change in antimicrobial susceptibility was observed: the percentage of strains susceptible to penicillin ($\text{MIC} \leq 0.08 \mu\text{g/ml}$) fell from 61 to 18 ($p < 0.0001$) and the percentage of resistant strains ($\text{MIC} \geq 1.2 \mu\text{g/ml}$) increased from 18 to 46. Among penicillin-resistant strains the proportion of penicillinase-producing strains (PPNG) was fairly constant (range 35-55%). The determination of susceptibility to antimicrobial agents performed locally allowed detection of approximately all PPNG strains whereas the increase in the occurrence of strains with chromosomally determined resistance was not revealed. The study comprised 70 PPNG strains of which 19% (13/70) carried the 7.4 kb Asian plasmid and 81% (57/70) the 5.3 kb African plasmid. None of these strains possessed the 38 kb conjugative plasmid, whereas it was found in 4.5% of the 376 non-PPNG strains available for plasmid analysis; 92% (410/446) of all strains had the small 4.2 kb plasmid and 5.4% (24/446) did not contain any plasmid. Overall, auxotype zero and proline-requiring strains were predominant, accounting for 53% (244/460) and 28% (131/460), respectively. In general, PPNG strains carrying the 5.3 kb plasmid were auxotype zero (49/57 = 86%) and those carrying the 7.4 kb plasmid were proline-requiring (9/13 = 69%).

Introduction

In many countries the management of gonorrhoea and other sexually transmitted diseases is based on syndrome related treatment algorithms.¹ An important measure to prevent spread of strains resistant to antibiotics currently in use is a continuous or sentinel surveillance of the in vitro antimicrobial susceptibility of recent clinical isolates of *N. gonorrhoeae*.^{2,3} The antimicrobial resistance of *N. gonorrhoeae* is rapidly changing, especially in areas where inefficient standard treatment regimens are applied.^{4,5}

The aim of the present study was to describe the evolution in antimicrobial resistance of *N. gonorrhoeae* in Dakar during the period 1982-1986 and to recommend methods suitable for local surveillance of antimicrobial resistance of *N. gonorrhoeae*.

The first part of the study was carried through in 1981-1983, during which period procedures for isolation, identification, transport and storage of *N. gonorrhoeae* was established at the Institut Pasteur in Dakar.⁶ It was in this context that the first penicillinase-producing *N. gonorrhoeae* (PPNG) strain was found in Dakar in 1981.⁷

Materials and methods

Neisseria gonorrhoeae strains

All gonococcal strains studied were isolated from patients attending three clinics in Dakar, Sénégal, 1982-1986:

Clinic A: The outpatients' clinic at the Institut Pasteur. The majority of patients of both sexes are Africans from lower or middle socio-economic classes.

Clinic B: military personnel, all young European men.

Clinic C: The outpatients' clinic at "Service de Dermatologie de l'Institut d'Hygiene Sociale". Only a few patients attending this clinic were included, except in 1984 when a larger group of female prostitutes were examined.

All isolates were identified, tested for penicillinase (β -lactamase) production and, except for 1982 strains, a preliminary antibiogram was determined in Dakar. The isolates were lyophilised and sent to the Institut Pasteur in Paris.

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At the Laboratoire des Neisseria, Institut Pasteur, Paris, the auxotypes were determined according to Catlin⁸ and plasmid analysis was performed on all PPNG strains. The strains were re-lyophilised and approximately 100 strains per year were sent to the Neisseria Department, Statens Seruminstitut, Copenhagen. All strains received at this laboratory were subjected to determination of susceptibility to antimicrobial agents and plasmid analysis (see below).

Antimicrobial susceptibility testing

Each strain was examined for susceptibility to penicillin, tetracycline and cefotaxime by the agar plate dilution method.⁹ The concentrations of antibiotics using two-fold dilution steps were as follows: sodium penicillin G: 0.01–9.6 µg/ml; tetracycline hydrochloride: 0.125–32 µg/ml; cefotaxime: 0.00025–1.024 µg/ml.

In addition, the susceptibility to penicillin, tetracycline, spectinomycin and thiamphenicol was determined by the agar disc diffusion test using 20 hours pre-diffusion.⁴ The disc content was: penicillin: 2.4 µg; tetracycline: 10 µg; spectinomycin: 100 µg; thiamphenicol: 30 µg. The production of β -lactamase was determined by the chromogenic cephalosporin test.¹⁰ The WHO *N. gonorrhoeae* reference strains A–D were included on each experimental day (22 experiments).

N. gonorrhoeae strains can be divided into three groups according to MIC of penicillin: susceptible: MIC \leq 0.08 µg/ml; less susceptible: MIC 0.15–0.60 µg/ml; resistant: MIC \geq 1.2 µg/ml. The definition of susceptibility is based on the clinical efficacy of standard treatment regimens.¹¹ Ninety five percent of uncomplicated urogenital infections with susceptible strains will be cured by penicillins given in standard dosages, whereas a high cure rate of infections with less susceptible strains can only be achieved by increasing the penicillin dosage. Infections with resistant strains require treatment with alternative drugs.

Table 1 *N. gonorrhoeae* strains isolated in Dakar 1982–86: prevalence of penicillinase-producing *N. gonorrhoeae* (PPNG)

	Number of strains isolated				
	1982	1983	1984	1985	1986
Clinic A					
Total	94	59	61	61	43
PPNG (%)	7 (7)	5 (8)	8 (13)	11 (18)	10 (23)
Clinic B					
Total	—	28	25	22	14
PPNG (%)	—	1 (4)	5 (20)	6 (27)	2 (14)
Clinic C					
Total	4	9	6	34	—
PPNG (%)	0	3 (33)	1 (17)	11 (32)	—
Total	98	96	92	117	57
PPNG (%)	7 (7)	9 (9)	14 (15)	28 (24)	12 (21)

Table 2 Penicillin susceptibility testing of non-penicillinase producing *N. gonorrhoeae*: Comparison of results obtained by the agar plate dilution method and the agar disc diffusion test (disc content 2.4 µg penicillin G)

Zone diameter (mm)	Number of strains at MIC (µg/ml)			
	0.01–0.08	0.15–0.60	1.2–9.6	Total
\leq 35	0	4	71	75
36–50	12	108	15	135
\geq 51	170	9	0	179
Total	182	121	86	389

Plasmid analysis

The analysis was performed according to the method of Birnboim¹² in Paris and of Takahashi and Nagano in Copenhagen.¹³

Statistical methods

Comparisons between frequencies are carried out using Fisher's exact test. Product-moment correlation coefficients (denoted *r*) are used to characterise the interdependence between MICs for different antibiotics and between MICs and zone diameters.

Results

Study population

The 460 gonococcal strains received in Copenhagen originated from 318 patients seen at clinic A (249 men and 69 women), 89 patients seen at clinic B (all men) and 53 patients seen at clinic C (19 men in 1982–84 and 34 women in 1985).

Prevalence of penicillinase-producing *N. gonorrhoeae* (PPNG)

As shown in table 1, a steady increase of PPNG infections was registered at clinic A, namely from 7% in 1982 to 23% in 1986. This change is statistically significant ($p = 0.01$). The changes seen at the other two clinics probably reflect the same evolution, but the figures are too small to be subjected to a detailed analysis. Overall 70 or 15% of the gonococcal strains recovered were PPNG.

Susceptibility to penicillin

A comparison of the results obtained for 69 PPNG and 389 non-PPNG strains by the agar dilution method (MIC in µg/ml) and the agar disc diffusion test (zone diameters in mm) was carried out (table 2). All PPNG strains showed maximum MICs of penicillin (not shown) and the inhibition zone for 67 out of 69 strains was zero whereas two strains showed a small zone (< 15 mm) in the experiments recorded. For 389 non-PPNG strains a strong correlation between MIC and zone diameter was found ($r = -0.93$) and 22% (86/389) had high level chromo-

somally mediated resistance to penicillin (MIC $\geq 1.2 \mu\text{g/ml}$); of these CMRNG (chromosomal mediated resistant *N. gonorrhoeae*) strains 11 had MICs $\geq 4.8 \mu\text{g/ml}$ and exhibited no zone of inhibition by the agar disc diffusion test.

The three levels of susceptibility to penicillin defined by means of MICs (see *Materials and methods*) can also be determined by the agar disc diffusion test. As shown in Table 2, 93% (170/182) of penicillin-susceptible strains had an inhibition zone diameter ≥ 51 mm, 89% (108/121) of less susceptible strains a zone diameter between 36 and 50 mm, and 83% (71/86) of resistant, non-PPNG strains a zone diameter ≤ 35 mm. None out of the 182 penicillin-susceptible strains showed zone diameters ≤ 35 mm and none out of the 86 penicillin-resistant strains showed zone diameters ≥ 51 mm. If these findings are used as a basis for definition of zone diameters corresponding to the three different levels of susceptibility, the classifications by the two methods disagree for 40 strains or about 10%, the strains being placed in groups adjacent to the correct one.

Susceptibility to tetracycline

A comparison of results obtained by the agar plate dilution method and the agar disc diffusion test using a disc containing 10 μg tetracycline hydrochloride was also performed. There was a strong correlation between the results obtained by the two methods ($r = -0.87$); 32% (149/458) were susceptible, 58% (265/458) were less susceptible (MIC = 1.0–4.0 $\mu\text{g/ml}$) and 10% (44/458) were resistant (MIC $\geq 8.0 \mu\text{g/ml}$) to tetracycline. For non-PPNG strains there was a strong correlation between MICs of penicillin and of tetracycline ($r = 0.80$). Susceptible (zone diameter ≥ 41 mm) and resistant (zone diameter ≤ 30 mm) strains were clearly recognised by means of the agar disc diffusion test. If these findings are used as a basis for definition of three levels of susceptibility to tetracycline by means of the agar disc diffusion test, it was found that 83 out of 458 strains or 18% showed a one-level discrepancy when a comparison with the corresponding MIC levels was made.

Susceptibility to cefotaxime

There was a strong correlation between MICs of penicillin and cefotaxime of non-PPNG strains ($r = 0.94$). The MIC of cefotaxime was within the range 0.005–0.512 $\mu\text{g/ml}$; out of the 86 CMRNG strains 32 (37%) showed a MIC of cefotaxime of 0.064 $\mu\text{g/ml}$ and 24 (28%) showed a MIC of cefotaxime of 0.128 $\mu\text{g/ml}$ or greater. The corresponding numbers (percentages) of the 70 PPNG strains were 4 (6%) and 3 (4%), respectively.

Table 3 Penicillin susceptibility of non-penicillinase producing *N. gonorrhoeae* strains isolated in Dakar 1982–86

Year	N	Percentage of strains at MIC of penicillin ($\mu\text{g/ml}$)		
		0.01–0.08	0.15–0.60	1.2–9.6
1982	91	66	22	12
1983	87	57	23	20
1984	78	38	36	26
1985	89	37	36	27
1986	45	22	47	31

Susceptibility to spectinomycin

All strains were susceptible to spectinomycin; 95% (437/460) showed a zone of inhibition within the limits 26–35 mm corresponding to MICs at $\leq 16 \mu\text{g/ml}$ (unpublished data). There was no difference between PPNG and non-PPNG strains, including CMRNG strains.

Susceptibility to thiamphenicol

In agreement with previous findings a positive correlation between susceptibility to thiamphenicol and to penicillin could be demonstrated in non-PPNG strains (data not shown). For thiamphenicol a strong correlation between MICs and zone diameter has been demonstrated previously (Spearman Rank correlation coefficient, -0.77).⁴

Changes in antimicrobial susceptibility of *N. gonorrhoeae* strains isolated in Dakar 1982–1986

Table 3 shows the percentage of non-PPNG strains at each level of MIC of penicillin during the period 1982–1986. The percentage of penicillin-susceptible strains fell from 66% in 1982 to 22% in 1986 ($p < 0.0001$) and during the same period the percentage of resistant strains increased from 12% to 31%. The figure illustrates changes in the prevalences of PPNG and CMRNG strains and of strains less susceptible and fully susceptible to penicillin. The increase in the prevalence of penicillin-resistant strains reflects a spread of PPNG as well as of CMRNG strains.

Comparison of results of antimicrobial susceptibility testing obtained in Dakar and in Copenhagen

Out of 272 strains that had been examined both in Dakar and Copenhagen, 72 had been recorded as penicillin-resistant in Dakar. Of these 72 strains 50 were designated PPNG; in Copenhagen 54 out of the 272 strains were found to be PPNG, that is, the recovery rate in Dakar was 93% (50/54). Out of the 272 strains 63 were CMRNG. In Dakar 22 were recorded as penicillin-resistant non-PPNG strains; only 11 of these 22 strains were actually CMRNG, which means that 83% (52/63) of CMRNG strains remained unrecognised.

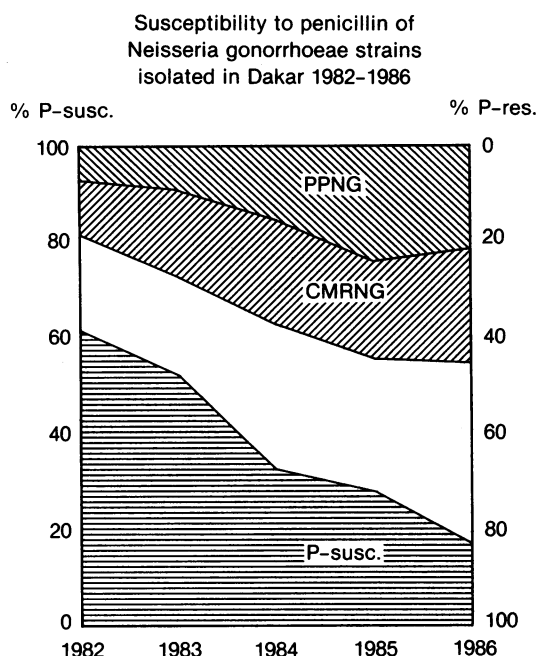


Figure Observed changes in the percentages of PPNG, CMRNG, and of gonococcal strains fully susceptible or less susceptible (blank area) to penicillin isolated in Dakar during the period 1982-1986.

Correlation between auxotype and susceptibility to penicillin

The proline-requiring and the proline plus arginine-requiring non-PPNG strains showed the same distribution according to susceptibility to penicillin, 48% and 54% of the strains being penicillin-resistant, respectively (table 4). These two groups were significantly more resistant than auxotype zero gonococci ($p < 0.0001$) and than arginine-requiring strains ($p = 0.007$); arginine-requiring strains and the mixed group of strains showed a distribution similar to that of auxotype zero strains. In general, auxotype zero PPNG strains carried the 5.3 kb plasmid (49/52 = 94%) and proline-requiring PPNG carried the 7.4 kb plasmid (9/11 = 82%).

Correlation between plasmid content and susceptibility to penicillin

The distribution according to susceptibility to penicillin for non-PPNG strains containing none ($N = 24$), the 4.2 kb ($N = 326$) or the 11.8 kb ($N = 3$) cryptic plasmids¹⁴ or the 4.2 plus the 11.8 kb plasmids ($N = 6$) were similar (table 5). Strains containing the 38 kb conjugative plasmid alone ($N = 9$) were more susceptible to penicillin than those carrying the small cryptic plasmid (4.2 kb) alone ($p = 0.002$) whereas those strains carrying both plasmids ($N = 8$) are more often CMRNG ($p = 0.028$).

Table 4 *N. gonorrhoeae* strains isolated in Dakar 1982-86: Correlation between auxotype and susceptibility to penicillin

Auxotype	Number of non-PPNG strains at MIC ($\mu\text{g/ml}$)			Total	PPNG	Total
	0.01-0.08	0.15-0.60	1.2-9.6			
Zero	116	67	9	192	52	244
Pro	25	38	57	120	11	131
Arg	16	1	3	20	0	20
Pro Arg	6	7	15	28	1	29
Others	20	8	2	30	6	36
Total	183	121	86	390	70	460

Prevalence of gonococcal strains containing the 5.3 kb, the 7.4 kb or the 38 kb conjugative plasmid

The study comprised 70 PPNG strains of which 19% (13/70) carried the 7.4 kb plasmid and 81% (57/70) the 5.3 kb plasmid. None of these strains possessed the 38 kb plasmid, whereas it was found in 4.5% (17/376) of non-PPNG strains; 92% (410/446) of all strains, including all PPNG strains, had the small 4.2 kb plasmid and 5.4% (24/446) did not contain any plasmid.

Correlation between auxotype and plasmid content

Results of auxotyping and plasmid analysis were available for 446 strains. Auxotype zero and proline-requiring strains were predominant (table 4); 86% (49/57) of PPNG strains carrying the 5.3 kb plasmid were auxotype zero and 69% (9/13) of PPNG strains carrying the 7.4 kb plasmid were proline-requiring. Arginine-requiring and arginine- plus proline-requiring strains were uncommon, and only one of these strains contained the 5.3 kb plasmid.

Discussion

The first isolate of penicillinase-producing *N. gonorrhoeae* (PPNG) in Africa was reported in 1979, and two years later, by 1981, reports from 23 African countries documented the presence of PPNG infections.¹⁵ There is indirect evidence that in certain areas the PPNG strains had emerged earlier, prob-

Table 5 Non-penicillinase producing *N. gonorrhoeae* strains isolated in Dakar 1982-86: Correlation between plasmid content and susceptibility to penicillin

Plasmid content	Number of strains at MIC ($\mu\text{g/ml}$)			Total
	0.01-0.08	0.15-0.60	1.2-9.6	
None	13	3	8	24
38 kb	9	0	0	9
11.8 kb	2	0	1	3
4.2 kb	149	109	68	326
4.2 kb + 38 kb	1	2	5	8
4.2 kb + 11.8 kb	4	1	1	6
Total	178	115	83	376

ably in 1976.¹⁶ During the following years, PPNG strains spread all over Africa, but with different trends; in Ibadan, Nigeria, for example, the prevalence of PPNG infection had reached 81% in 1984,¹⁵ whereas in the present study from Dakar, Sénégal, the rate reached only 23% up to the year 1986. In a semirural community in Gabon, PPNG infections accounted for 7% of cases of gonorrhoea in 1981, 48% in 1984 and then only 23% in 1985.¹⁷

At the emergence of PPNG strains, two distinct types of strains were recognised: one type contained a 5.3 kb β -lactamase-coding plasmid, did not contain the 38 kb transfer plasmid, required arginine (Arg⁻) for growth, was susceptible to tetracycline and epidemiologically linked to West Africa; another type contained a 7.4 kb β -lactamase-coding plasmid, and, in addition, around 40% contained the 38 kb transfer plasmid; these strains were auxotype zero (wild type) or proline-requiring, tetracycline-resistant, and epidemiologically linked to the Far East. Since then, the β -lactamase-coding plasmids have spread among different gonococcal auxotypes and among strains with different antimicrobial susceptibility patterns;¹⁸⁻²⁰ the 38 kb transfer plasmid has been found also in strains carrying the 5.3 kb plasmid and in varying proportions of non-PPNG strains. The spread of β -lactamase-coding plasmids seems to be independent of the presence of the 38 kb plasmid, and this latter plasmid has spread rapidly in certain gonococcal populations.²¹

In Dakar the predominant type of PPNG during the period 1982-1986 was auxotype zero carrying the 5.3 kb plasmid, of which one carried the 38 kb plasmid. Overall, auxotype zero (53%) and proline-requiring (28%) strains were predominant, and strains that required arginine or arginine as well as proline were uncommon (4% and 6%, respectively). The 38 kb plasmid was detected in a few strains every year and showed no tendency to spread; in total, this plasmid was only found in 4.5% of the non-PPNG strains studied. This finding is in contrast to the results of a contemporary study, in which the prevalence of the 38 kb plasmid in isolates from Greenland was found to increase from 0% to 70% (Reimann K and Lind I: unpublished data), Knapp has reported on a similar rapid spread in some areas of the U.S.A.²¹

The spread of penicillin-resistant non-PPNG (CMRNG) strains in Africa has not been studied as closely as the spread of PPNG. In the report from Gabon,¹⁷ the percentage of isolates for which the MIC of tetracycline was greater than 1 μ g/ml increased from 5% in 1981 or 1984 to 28% in 1985; this finding may reflect an increased importation of CMRNG strains, for example from the Far East, although the MIC of penicillin for 90% of isolates passed from 2 μ g/ml in 1981 to 0.5 μ g/ml in 1985. In our study, a significant change in antimicrobial sus-

ceptibility of non-PPNG-strains was observed: the percentage of CMRNG strains increased from 12 in 1982 to 31 in 1986, and the percentage of susceptible strains fell from 66 to 22 ($p < 0.0001$). The possibility of detecting these changes by antimicrobial susceptibility testing performed locally was assessed. The local performance allowed detection of approximately all PPNG strains, whereas the increase in the occurrence of strains with chromosomally determined resistance was not revealed. In retrospect, details of the agar disc diffusion test used, conditions for storage of discs, definitions used for the designations r (resistance) versus s (susceptible), etc. could not be cleared up adequately. It is considered most likely that inappropriate storage conditions of the penicillin-containing discs were the reason why a high proportion of CMRNG strains remained unrecognised.

In the reference laboratory in Copenhagen, the results obtained by the agar plate dilution method and by agar disc diffusion tests were in agreement, and both methods allowed a current assessment of changes in antimicrobial pattern. Each of the 22 experiments included the WHO *N. gonorrhoeae* reference strains A-E. Strain E is a PPNG. There was a strong correlation between MIC and zone diameter, and the four different levels of penicillin susceptibility defined by the reference strains A-D could be identified by both methods without any overlapping. The study from Gabon included the WHO reference strains A-E in each series of MIC determinations (14 experiments). The different levels of susceptibility to penicillin (and other antibiotics) were correctly identified, and only 4.2% (14 of 336) of the determinations differed by more than one dilution from the median. Therefore, the above-mentioned results obtained in two studies of African strains are directly comparable and provide an example of the benefit of using reference strains.

In general, disc diffusion tests are performed without pre-diffusion, that is, without establishing a concentration gradient of the antibiotic into the medium before inoculation; regarding β -lactam antibiotics and tetracycline, discs with higher contents than those employed in the present study are commercially available and therefore most often used. Under these conditions, the differences between diameters of inhibition zones for susceptible, less susceptible and resistant strains decrease, especially if the medium is unfavourable to growth of gonococci (Lind I and Bentzon M W: unpublished data). If the observed differences for the reference strains are less than the expected day-to-day variation, the disc diffusion test is of no use. When classification into three groups is made on the basis of results obtained by two different methods, complete agreement cannot be expected in spite of the strong correlation found between the two sets of results. The findings of 90%

(penicillin) and 82% (tetracycline) agreement between MICs and zone diameter, and the fact that none of the susceptible strains were categorised as resistant and vice versa, indicate that the agar disc diffusion method is useful.

Snell and Brown²² organised an external quality assessment of antimicrobial susceptibility testing of *N. gonorrhoeae* comprising 411 United Kingdom laboratories. Detection of PPNG was achieved by almost 100% of the laboratories, whereas the detection of CMRNG proved more difficult. They demonstrated that greater error rates were associated with the use of high content discs for testing tetracycline (25, 30 or 50 µg versus 2, 5 or 10 µg) and with the use of low content discs for testing spectinomycin (25 or 30 µg versus 100 µg). Unfortunately, an insufficient number of laboratories used cefuroxime discs other than 30 µg to allow comparison to be made. There was no difference in error rates using the following low contents of penicillin: 1, 1.5 and 2 units (0.6, 0.9 and 1.2 µg) per disc. These results are in complete agreement with those obtained in our laboratories. The recent comprehensive study from the USA²³ on the standardisation of antimicrobial susceptibility testing of *N. gonorrhoeae* focuses on lot to lot variations for commercially available discs (one high content disc per antibiotic) and for components of the media, and on the stability of antibiotics in agar plates. The interpretive criteria recommended for the agar disc diffusion test were derived from results using high content discs and are thus not comparable with those obtained in the present study. The importance of the choice of medium for the antimicrobial susceptibility testing of *N. gonorrhoeae* has also been emphasised by Gill and Ison,²⁴ who included the WHO as well as other reference strains in their study.

As discussed by Judson,²⁵ the guidelines "Antibiotic-Resistant Strains of *Neisseria gonorrhoeae*, Policy Guidelines for Detection, Management, and Control" published by Centers for Disease Control in the USA²⁶ present extremely complex and detailed recommendations which in certain cases lack supporting data. This is also true for the section on determination of antimicrobial susceptibility. Reservations on recommendations based on limited data are clearly stated in the preamble of the recommendations. For that reason, the obvious discrepancies between our conclusions and those from the CDC will not be discussed in detail.

Our study has confirmed that the use of well-defined reference strains is a valuable tool in the standardisation of antimicrobial susceptibility testing of gonococci. It has also shown that it is possible to identify accurately the various levels of antibiotic susceptibility of *N. gonorrhoeae* by means of the agar disc diffusion test. In other words, the agar disc

diffusion test can be standardised and used as an equivalent to the agar plate dilution test in the surveillance of the antimicrobial susceptibility of *N. gonorrhoeae*.

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